DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - ~~SHRIMP~~ MYSID

1. **CHEMICAL:** S)-3,5-dihydro-5-methyl-2-methylthio -5-phenyl-3-phenylamino-4H-imidazol-4-one — PC Code No.: 046679

2. **TEST MATERIAL:** Fenamidone (RPA 407213) — Purity: 99.8%

3. **CITATION:**

Author: Sousa, J.V.

Title: RPA 407213 - Acute toxicity to mysids (*Mysidopsis bahia*) under flow-through conditions

Study Completion Date: June 11, 1999

Laboratory: Springborn Laboratories, Inc.
790 Main Street
Wareham, Massachusetts 02571-1075

Sponsor: Rhône-Poulenc Ag Company, 2 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709

Laboratory Report ID: 98-9-7477

MRID No.: 45385721

DP Barcode: D275213

4. **REVIEWED BY:** Mary Thomas, Staff Scientist, Dynamac Corporation

Signature: Mary Thomas **Date:** 1/29/02

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: [signature] **Date:** 1/29/02

5. **APPROVED BY:** [redacted], Biologist, OPP/EFED/ERB III

Signature: James J. Goodyear, Ph.D.
US EPA, Mail Code 7507C
1200 Pennsylvania Ave. NW
Washington, DC 20460
James Goodyear **Date:** 5/2/2

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Mysidopsis bahia*

Age or Size of Test Organism: <24 hours old

Definitive Test Duration: 96 hours

Study Method: Flow-through

Type of Concentration: Mean measured

7. CONCLUSIONS:

In this 96-hour acute LC_{50} test with an estuarine/marine organism, mysid shrimp, *Mysidopsis bahia*, were exposed to Fenamidone (RPA 407213) at mean measured concentrations of 19, 29, 47, 82 and 128 µg a.i./L. Nominal concentrations were 17, 28, 47, 78 and 130 µg a.i./L (mean measured concentrations were 98-110% of nominal). Sublethal effects (lethargy and loss of equilibrium) were observed in the 82 µg a.i./L treatment level at 48, 72 and 96 hours. Complete mortality (100%) was observed in the 128 µg a.i./L treatment levels at 48, 72 and 96 hours. **The 96-hour LC_{50} and NOEC values were 69 µg a.i./L and 47 µg a.i./L, respectively. Based on these results, RPA 407213 is categorized as very highly toxic to *Mysidopsis bahia*.**

This study is scientifically valid and fulfills the requirements of an acute LC_{50} test with an estuarine/marine organism (Subdivision E, §72-3(C) [shrimp]). **This study is classified as CORE.**

Results Synopsis

24-Hour:
LC_{50}: >130 µg a.i./L 95% C.I.: N/A

48-Hour:
LC_{50}: 76 µg a.i./L 95% C.I.: 47-130 µg a.i./L

72-Hour:
LC_{50}: 72 µg a.i./L 95% C.I.: 47-130 µg a.i./L

96-Hour:
LC_{50}: 69 µg a.i./L 95% C.I.: 47-82 µg a.i./L
NOEC: 47 µg a.i./L

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. Temperature was maintained at 25 ± 1°C throughout the study period, instead of the guideline requirement of 22 ± 1°C.

2. Biomass loading rate was not reported.

11. SUBMISSION PURPOSE: This study was submitted to provide data for the registration of RPA 407213 on its toxicity to estuarine/marine mysid shrimp.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Mysidopsis bahia*
Age Juvenile (≤ 24 hours old) mysids should be used	<24 hours old

Guideline Criteria	Reported Information
Supplier	Laboratory cultures established at Springborn with brood stocks obtained from Aquatic BioSystems, Inc., Ft. Collins, Colorado
All mysid are from same source?	Yes
All mysid are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	14
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Fed ≤48 hours old live brine shrimp (*Artemia salina nauplii*) twice daily during testing.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Natural filtered seawater
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) mysid and 10-17 ‰ for estuarine (euryhaline) mysid, weekly range <6 ‰	31-32‰
Water Temperature Approx. 22 ± 1 °C	25 ± 1°C
pH 8.0-8.3 for marine (stenohaline) mysid, 7.7-8.0 for estuarine (euryhaline) mysid, monthly range < 0.8	7.7-7.8
Dissolved Oxygen Between 60 and 105% saturation. If needed, aerate prior to introduction of chemical.	4.3-5.7 mg/L
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	<2.0 mg/L

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. shrimps and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	1. Glass 2.30 × 15 × 20 cm 3. 8-11 L
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent-flow proportional diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6.5 aquarium volume additions/day
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day (N/A for shrimps)	Not reported
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Dimethylformamide (0.096 mL/L)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 mysid, then no definitive test is required.	A range finding study was performed under flow-through conditions with a negative control and five nominal test concentrations of RPA 407213 (17, 28, 47, 78 and 130 µg a.i./L). By 96 hours 90% and 100% mortality was observed in the 78 and 130 µg a.i./L treatment levels, respectively. Lethargy was observed in all the mysids exposed to 78 µg a.i./L treatment level.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	Dilution water control, solvent control, and 17, 28, 47, 78 and 130 µg a.i./L
Number of Test Organisms Minimum 20/level, may be divided among containers	20/level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Measured daily in each aquarium and continuously in one test vessel. 2. Measured daily.

Guideline Criteria	Reported Information
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Analytical determination of test substance from the definitive test was performed on samples from test vessel at the beginning and end of the test.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	97.8-105%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No mortality for control
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (µg/L)		Number of Mysid	Mean cumulative mortality (%)			
			Hours of Study			
Nominal	Mean Measured		24	48	72	96
Control	<4.8	20	0	0	0	0
Solvent	<4.8	20	0	0	0	0
17	19	20	0	0	0	0

Concentration (µg/L)		Number of Mysid	Mean cumulative mortality (%)			
			Hours of Study			
Nominal	Mean Measured		24	48	72	96
28	29	20	0	0	0	0
47	47	20	0	0	0	0
78	82	20	0	60	70	80
130	128*	20	40	100	100	100

*The mean measured table (p. 72) shows that the highest mean measured concentration was 128 µg/L instead of 130 µg/L, which was reported by the study author.

Sublethal effects (lethargy and loss of equilibrium) were observed in the 82 µg a.i./L treatment level at 48, 72 and 96 hours. Complete mortality (100%) was observed in the 130 µg a.i./L treatment levels at 48, 72 and 96 hours.

B. Statistical Results

Statistical Method: A computer program (Stephan, 1982) determined the LC_{50} values and 95% confidence intervals using either moving average angle analysis, probit analysis, or nonlinear interpolation (binomial probability). Selection criteria included the establishment of a dose-response relationship, the number of concentrations causing partial responses, and the span of responses bracketing the LC_{50} value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence limit was selected.

The nonlinear interpolation method (binomial probability) was used to determine the following values:

24-Hour:
LC_{50}: >128 µg a.i./L 95% C.I.: N/A

48-Hour:
LC_{50}: 76 µg a.i./L 95% C.I.: 47-128 µg a.i./L

72-Hour:

LC_{50}: 72 µg a.i./L 95% C.I.: 47-128 µg a.i./L

96-Hour:
LC_{50}: 69 µg a.i./L 95% C.I.: 47-82 µg a.i./L
NOEC: 47 µg a.i./L (based on mortality, lethargy and loss of equilibrium)

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} value and 95% confidence intervals were estimated using nonlinear interpolation (bionomial probability) via ToxAnal software. The NOEC was determined visually because there was no mortality in the control groups and the distinction between concentrations eliciting an effect (mortality and sublethal effects) or no effect was clear.

24-Hour:
LC_{50}: >128 µg a.i./L 95% C.I.: N/A

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NOEC: 47 µg a.i./L (based on mortality, lethargy and loss of equilibrium)

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those reported by the authors. The 96-hour LC_{50} was 69 µg a.i./L. Based on these results, RPA 407213 is categorized as **very highly toxic** to *Mysidopsis bahia.* The NOEC was 47 µg a.i./L, based on mortality, lethargy and loss of equilibrium.

There were some deviations from US EPA protocol. These included, higher water temperature (25 ± 1°C) than required (22 ± 1°C), and the failure to report the biomass loading rate required by US EPA. These deviations were not considered to have significantly impacted mysid response or test conditions. This study is classified as Core.

The study author reported the highest mean measured concentration as 130 µg a.i./L in the

text and tables of the study report; however, on p. 72 of the study report, the highest mean measured concentration is 128 µg a.i./L.

This study was conducted in accordance with USEPA Good Laboratory Practice Regulations. A Quality Assurance Statement was provided.

16. REFERENCES

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